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(54) MONOMERIC AND DIMERIC ANTIBODY-FRAGMENT FUSION PROTEINS

FUSIONSPROTEINE VON MONOMEREN UND DIMEREN VON ANTIKÖRPERFRAGMENTEN

PROTEINES FUSIONNEES MONOMERES ET DIMERES A FRAGMENTS D'ANTICORPS

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- **PROTEIN ENGINEERING vol. 4, no. 4, April 1991, ENGLAND GB pages 457 - 461 BLONDEL AND BEDOUELLE 'Engineering the quarternary structure of an exported protein with a leucine zipper' s**
- **BIOCHEMISTRY vol. 31, no. 6, 18 February 1992, EASTON, PA US pages 1579 - 1584 PACK AND PLÜCKTHUN 'Miniantibodies: Use of amphipatic helices to produce functional, flexibly linked dimeric Fv fragments with high avidity in Escherichia coli's**

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Description

The present invention describes a new class of antigen binding molecules which contain Fv-fragments of an antibody but do not use the constant antibody domains. They can also dimerize with other antibody fragment molecules or with non-antibody fragment molecules to form bi- or multifunctional antibody-fragment fusion proteins and so-called mininantibodies, respectively. The new fusion proteins can be used in the broad field of diagnostic and therapeutical medicine.

Background of the Invention

Since a few years there is a great interest in the biotechnological field to modify naturally occuring antibodies in order to obtain more specified and more individual antibody species. Therefore, attempts have been made to produce (modified) antibody fragments.

All naturally occuring antibodies of all classes have at least two binding sites. This enables them to bind to a surface with a greater functional affinity (also called avidity) than monovalent fragments, such as Fab fragments. Over the last few years, methods have been described (Skerra and Plückthun, 1988, Science 240, 1038-1040 ; Better et al., 1988, Science 240, 1041-1043) with which functional antibody fragments can be produced in Escherichia coli. These include the Fv fragment (the heterodimer consisting of V_H and V_L) and the Fab fragment (consisting of the complete light chain with the domains V_L and C_L as well as the first two domains of the heavy chains V_H and C_{H1}).

The Fv fragment, however, has a tendency to dissociate into V_H and V_L and therefore, it is advantageous to link the two domains covalently. One particular way of linking them is by designing a peptide linker between them, either in the orientation V_H-linker-V_L or V_L-linker-V_H (Bird et al.,1988, Science 242, 423; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85, 5879) The resulting fragments are called single-chain Fv fragments.

All these fragments are, however, monovalent. We describe in this invention a method to engineer small dimerization domains based on peptides forming amphipathic helices. These peptides will be referred to as "intercalating", but this term is only meant to express the ability of targeted association and not a restriction referring to a particular structure of the dimerization interface.

While the methodology described here, is in principle applicable to either Fab, Fv or scFv fragments, it is the latter for which their use is most advantageous. In this case bivalent fragments can be constructed cf very small size, and still the dissociation into V_L and V_H as well as the wrong matching of fragment chains, e.g. V_L-V_L, can be prevented.

Antibody fragments of small size are of particular advantage in many applications. In diagnostic applications (e. g. ELISA, RIA, etc.), the smaller molecules surface decreases the problems of nonspecific interactions, which are known to frequently involve the constant domains. The same is true in using antibody fragments as ligands in affinity chromatography. In tumor diagnostics or therapy, it is important that a significant proportion of the injected antibody penetrates tissues and localizes to the tumor, and is dependend on the molecular dimensions (Colcher et al., 1990, J. Natl. Cancer Inst. 82, 1191-1197). Expression yields and secretion efficiency of recombinant proteins are also a function of chain size (Skerra & Plückthun, 1991, Protein Eng. 4, 971) and smaller proteins are preferred for this reason. Therefore, molecules of a small size are advantageous for several reasons.

Previously, decreasing the molecular dimensions of the antibody meant the preparation of proteolytic fragments. The smallest bivalent fragments, $(Fab)'_2$ fragments, are still about twice the size of the present fragments of this invention. Therefore, these new fragments combine three features: (a) small size, (b) bivalence or bifunctionality and (c) ability of functional expression in E. coli.

There is great interest in bifunctional antibodies in a large number of areas. Bifunctional antibodies may be defined as having two different specificities for either two different antigens or for two epitopes of the same antigen.

There are currently a number of methods how to produce bifunctional antibodies. However, none of the existing methods allows to produce exclusively bifunctional antibodies in vivo, but rather a mixture of molecular species always occur, requiring complicated and expensive separation procedures.

Four principal methods can be distinguished. In the first, chemical crosslinking is used, which may advantageously use heterobifunctional crosslinkers. By this method, whole antibodies (Staerz et al., 1985, Nature 314, 628; Perez et al, 1985, Nature 316, 354-356), Fab fragments (Carter et al., 1992, Biotechnology 10, 163) and scFv fragments (Cumber et al., 1992, J. Immunol. 149, 120) have been chemically crosslinked after purification.

The second previous method involved the fusion of two hybridomas to give a so-called heterohybridoma or "quadroma". In this method, any light chain can pair with any heavy chain, and the two heavy chains can give homodimers or heterodimers resulting in very complicated product mixtures (Milstein & Cuello, 1983, Nature 305, 537).

The third method is related to the second and consists of transfecting two expression plasmids into a hybridoma cell, encoding the heavy and light chain of the second antibody (Lenz & Weidle, 1990, Gene 87, 213) or a retroviral vector (De Monte et al., 1990, Acad. Sci. 87, 2941-2945). However, once introduced, the product mixture is identical as in the second procedure.

Finally, antibodies have been reduced, mixed and reoxidized (Staerz & Bevan, 1986, Immunology Today 7). Again, very complicated product mixtures are obtained requiring sophistical separation and quality control procedures.

Thus a method is still needed allowing the isolation of exclusively heterodimeric antibodies directly without the complicated preparation required from chemical crosslinking. In the present invention, this problem is solved by (i) covalently linking corresponding VH and VL domain in a scFv fragment and (ii) using dimerization domains only allowing the formation of heterodimers, such as certain leucine zippers and derivatives.

Another important consideration in the present invention was the desire to make the MW of the bispecific antibody as small as possible for reasons explained above in detail. This was achieved by using scFv fragments.

A number of uses of bispecific antibodies bave been described, and most of them would benefit from this new technology. For example, bispecific antibodies are of great interest in tumor therapy. One arm of the antibody may bind to a tumor marker, the other arm to a T-cell epitope, a toxin, or a radionuclide binding peptide or protein to bring a killing function close to the tumor cell. In diagnostics, one arm may bind to the analyte of interest and the other to a principle which can easily be quantified, e. g. an enzyme. Finally, in cellular applications, it may be advantageous to obtain higher selectivity in binding, if two different epitopes or the same protein complex can be recognized or if two different proteins can be recognized on the same cell surface.

Thus, it was object of the invention to create new individual and stable antibody fragment fusion proteins with bi- or even mulitfunctional binding sites.

It has been found that antibody fragment fusion proteins containing Fv-fragments could be produced by genetic engineering methods which show specified and improved properties.

Object of the invention is, therefore, a monomeric antibody-fragment fusion protein essentially consisting of a Fv-fragment of an antibody and a peptide which is capable to dimerize with another peptide by noncovalent interaction.

The term "noncovalent interaction" means every existing under normal condititions stable linkage which is not related to a covalent binding, for example linkage by Van der Waal's forces, (steric) interdigitation of amphiphilic peptides, especially peptide helices, or peptides bearing opposite charges of amino acid residues. The correspondingly effective peptides are called above and below interactive or intercalating peptides.

The amphiphilic peptides consist of up to 50 amino acids. Preferrably they consist of 10 to 30 amino acids. In a preferred embodiment of the invention the interactive peptide is a peptide helix bundle (comprising of a helix, a turn and another helix, see above). In another embodiment the interactive peptide is a leucine zipper consisting of a peptide having several repeating amino acids, in which every seventh amino acid is a leucine residue. In other cases according to the invention the peptide bear positively or negatively charged residues, e.g. lysine (positively charged) or glutamic acid (negatively charged) in a way that this peptide can bind to another peptide (of a second monomeric unit) bearing opposite charges.

The Fv-fragment and the intercalating peptide are linked together either directly or by a linker peptide, preferrably by a linker peptide. In a preferred embodiment the linker peptide is a hinge region sequence of an antibody.

As defined, the Fv-fragment consists of the V_L and V_H region of an antibody. The Fv-fragment according to the invention is preferrably a single chain fragment. Single chain fragments can be obtained by standard techniques using standard linker molecules.

Furthermore, object of the invention is a dimeric fusion protein essentially consisting of two monomeric fusion proteins, wherein the linkage of the monomeric units bases on noncovalent interaction of identical or different peptides, characterized in that at least one monomeric unit is an antibody-Fv-fragment fusion protein as defined above.

If the dimer contains two Fv-fragments, the Fv-fragments may be the same (identical antigen binding sites) or may be different (different antigen binding sites). In these cases mono- and bispecific (Fv)- miniantibodies can be obtained. According to the invention bispecific mini-antibodies are preferred.

The interactive peptides may be the same or may be different; preferrably they are identical. The intercalating peptides may be associated in parallel or in antiparallel fashion.

Object of the invention is, therefore, above all, a dimeric fusion protein consisting of two Fv-fragments with different specificity (antigen binding sites) and identical intercalating helix peptides, the antibody fragments and the peptides are linked together by a hinge region sequence.

Furthermore, object of the invention is a dimer consisting of a monomeric unit containing a Fv-fragment and another monomeric unit wherein the Fv-Fragment was replaced by a non-antibody peptide. The non-antibody peptide may be a toxin, like ricin, a chelator- or metal binding peptide, or an enzyme (e.g. marker enzyme), or a peptide bearing a detectable lable (e.g. a radioisotope).

The non-antibody peptide can also bear a corresponding binding site for said groups, binding sites directed to T-cells or T-cell fragments included.

Furthermore, the invention relates to monomers and dimers, as defined above, wherein the interactive peptide(s) is (are) additionally fused at the C-terminus to target proteins/peptides as mentioned above, the corresponding binding sites included. Thus, the resulting fusion proteins and miniantibodies, respectively, are multifunctional.

The invention relates, furthermore, to a process for preparation of a monomeric antibody fusion protein as defined

above, characterized in that the genes coding for the Fv-fragment, the interactive peptide and, if desired, the linking peptide are cloned into one expression plasmid, a host cell is transformed with said expression plasmid and cultivated in a nutrient solution, and the monomeric fusion protein is expressed in the cell or secreted into the medium.

Object of the invention is, finally, a process for preparation of a dimeric fusion protein as defined above, characterized in that the genes coding for the complete monomeric fusion proteins or parts of it are cloned at least into one expression plasmid, a host cell is transformed with said expression plasmid(s) and cultivated in a nutrient solution, and either the complete dimeric fusion protein is expressed in the cell or into the medium, or the monomeric fusion proteins are separately expressed and the noncovalent linkage between the two monomeric units is performed in the medium or in vitro, and in the case that only parts of the fusion proteins were cloned, protein engineering steps are additionally performed according to standard techniques.

The dimeric Fv-fragments containing fusion proteins according to the invention show a high avidity against corresponding antigens and a satisfying stability. These novel bivalent or bifunctional molecules can be prepared as folded and assembled molecules in E. coli. These miniantibodies are compatible with functional expression by secretion.

Detailed description of the invention

The oligomerization domains were selected for having a fairly small molecular weight and for being compatible with transport of the fusion protein through the membrane. They are based on two different types of amphiphilic helices.

Amphiphilic helices are known to predominantly, but not exclusively, associate in two different molecular structures: Four helix bundles and coiled coils. The design and formation of helix bundles has been studied previously (Eisenberg et al., 1986, Proteins 1, 16-22; Ho and deGrado, 1987, J. Am. Chem. Soc. 109, 6751-6758; Regan and deGrado, 1988, Science 241, 976-978 ; Hill et al., 1990, Science 294, 543-546). This molecule association is also known from natural proteins (Richardson, 1981, Adv. Prot. Chem. 34, 167).

The four helix bundle may be formed from either four separate molecules (each contributing one helix), two molecules containing two helices each (connected as helix-turn-helix) or one molecule containing a helix-turn-helix-turn-helix-turn-helix motif. For dimerization or multimerization, only the first two are suitable.

Three variations of this latter theme were tested. In the first, one helix of the sequence given in Eisenberg et al. (1986) (Proteins 1, 16-22) was used. In the second, this sequence was extended by a small hydrophilic peptide ending in a cysteine. Once the helices are associated, the hydrophilic peptides are held in sufficiently close contact that they can collide and a disulfide bond can form under oxidizing conditions, as in the periplasm of E. coli. In the third variation, two helices are used in tandem, separated by a short turn encoding peptide.

In the second design, peptides are used which can form so-called coiled-coil structures. Such peptides occur in transcription factors such as e. g. GCN4 from yeast and have been called leucine zippers (Landschulz et al., 1988, Science 240, 1759-1764). The crystal structure of this has been solved recently (O'Shea et al., 1991, Science 254, 539-544) and showed a parallel arrangement of the helices.

A covalent attachment of the helices is possible by a small peptide extension, again containing a cystein. Since the helices are now parallel, the peptide extension can be much shorter, since the distance is much smaller.

The various dimerization devices (intercalating helices) were however not fused to the antibody domain directly. It is advantageous to introduce a flexible peptide between the end of the scFv fragment and the beginning of the helix. As an example, the upper hinge region of mouse IgG3 has been used. However, a variety of hinges can be used. It is not required for dimerization per se, but provides a spacing of the two scFv domains similar to the antigen binding sites of a whole antibody. This way, the two binding sites span a greater distance in space and therefore can reach neighboring antigens on a solid surface.

The naturally occuring hinges of antibodies are preferred embodiments of hinges in bivalent miniantibodies. In the case of bifunctional miniantibodies, the hinges may be shorter, since frequently molecules from different surfaces are to be crosslinked as close as possible, and flexibilty of the dimer is not necessary. The choice of the hinge is governed by the desired residue sequence, l ength (Argos, 1990, J. Mol. Biol. 211, 943-958), compatibility with folding and stability of the amphiphilic helices (Richardson & Richardson, 1988, Science 240, 1648-1652), secretion and resistance against proteases .

The present invention deals with peptides as dimerization devices, which should be as small as possible. One preferred embodiment is the use of peptides which can form amphipathic helices. Such helices shield the hydrophobic surface by dimerization or even multimerization. Helices of this type are characterized by their having hydrophobic patches on one face of the helix, and containing a sufficient number of helix-forming residues. Rules for such peptides are discussed in Eisenberg et al. 1986, O'Shea et al., 1991 (Science 254, 539-544),1992 (Cell 68, 699-708).

Natural peptides of this type are found as the so-called leucine zippers, characterized by a periodic occurence of leucine (every seventh residue) and other hydrophilic residues (e. g. valine) also every seventh residue. As these priniples are now understood (O'Shea et al. 1991, 1992, literature cited), the sequence can be varied to incorporate residues which make the association of homodimers unfavorable, but favors the association of heterodimers. Such

sequence alteration can e. g. involve the incorporation of charge bridges, such that in the homodimers, like charges repel each other and in the heterodimer, opposite charges attract each other (see below).

The present invention can also be extended to bifunctional miniantibodies. In this case, dimerization devices (intercalation peptides) have to be used which will only allow the formation of heterodimers, but not homodimers. A preferred embodiment of this part of the invention are two different coiled-coil helices, such as in naturally occuring leucine zippers, e. g. from the transcription factor proteins jun and fos (O'Shea et al., 1989, Science 245, 646-648).

In a further embodiment of the invention, the constant scFv-hinge-helix can be extended at the C-terminus to result in a fusion protein. For example, a fusion to an enzyme may be made to use such bivalent constructs in diagnostics. Such enzymes are e. g. alkaline phosphatase, luciferase or horse radish peroxidase. The advantage of such a antibody-enzyme fusion protein would be that the bivalence of the antibody would lead to an enhanced binding to the surface-bound antigen. The advantage over a fusion protein prepared by conventional technology (i. e. chemical coupling of the antibody to the enzyme of choice) would be a greater batch-to-batch consistency; homogeneity of the product and the much simpler method of preparation, namely from E. coli in a single step.

In the same fashion, the miniantibodies may be extended at the C-terminus to incorporate a toxin. Such immunotoxins would be bivalent or even bispecific and thus combine the advantages of such antibody fragments linked above with the advantages in tumor therapy known for immunotoxins. Similarly, a metal binding peptide or protein could be linked genetically to be used in radioimmunotherapy or in tumor imaging. The same advantages for any genetically encoded hybrid protein hold true as given above for the antibody-enzyme fusions.

In another embodiment of the invention, a construct of the type scFv-hinge-helix may be made to dimerize with another protein fused to a dimerization domain, in complete analogy as described above for the formation of bispecific miniantibodies. In this fashion, the scFv fragment would e. g. be fitted with the helix of the fos protein. Such foreign protein, which could be made to form heterodimers with the scFv fragment, include enzymes useful in diagnosis, toxins, metal-binding peptides or proteins useful in radioimmunotherapy or radio-imaging.

Using the principles of this invention, the dimerization domains presented here can also serve for purification purposes. A recombinant protein of any kind can be fused to a dimerization domain, e.g. to hinge-fos-zipper. After coexpression with a scFv-hinge-jun, the heterodimer can be purified in one step with an affinity column for the scFv-specificity. In an alternative approach, the 'opposite' zipper, linked to a column support, 'catches' the protein-hinge-zipper when passing through the column as a crude cell extract.

The elution of the pure fusion protein from the column is possible using the unfolding temperature of the zipper. A subsequent separation from the dimerization domain is achievable by introduction of a proteolytic site, e.g. for blood clotting factor Xa, into the hinge (Nagai & Thogerson, 1987, Meth. Enyzmol. 152,,461-481).

A particular advantage of the miniantibodies described in this invention is the ability to assemble functionally in Escherichia coli. In the case of homobivalent constructs, a dimerization principle is used which allows the formation of homodimers. Examples described above include the coiled-coil helix (leucine zipper) of the yeast protein GCN4 or the helices from an antiparallel 4-helix bundle. In this case, the scFv fragment is expressed in the presence of a bacterial signal sequence and carries at the end of the gene of the scFv fragment the codons for a hinge and the dimerization helix or helix-turn-helix. The helices are compatible with secretion to the periplasmic space in E. coli, where protein folding, disulfide formation and assembly occurs. Under these conditions, the homodimeric proteins form by themselves and can directly be isolated in the dimeric form.

If heterobivalent constructs are desired, two different scFv fragments or one scFv fragment associating with a different protein need to associate. In the preferred embodiment of this invention, both proteins to be assembled are expressed in the same cell, preferably on the same plasmid, preferably as a dicistronic operon. The design of artificial dicistronic operons is explained e.g. in Skerra et al. (1991, Protein Eng. 4, 971). Since the assembly must take place in the periplasm, because the scFv fragment can only fold in the oxidizing milieu, both proteins must be transported and both must be fitted with a signal sequence. The dimerization peptides must be chosen such that they promote the association of two different proteins, but prevent the association of the respective homodimers. Examples of such proteins are the leucine zipper peptides of the proteins fos and jun (see above).

When not expressed in the same cell, the different scFv-hinge-zipper constructs have to be mixed together as a crude cell extract or purified protein and treated with raised temperature. In absence of the 'opposite' zipper, e.g. a scFv-hinge-jun-zipper construct is able to form homodimers. After short heating to the melting temperature of around 40~C, the zippers of the unwanted homodimer unfold and form a much more stable heterodimer (O'Shea et al., 1992, Cell 68, 699-708). Without raising the temperature, formation of heterodimers in vitro is not possible, as tested in experiments.

Short Description of the Figures and the Sequence Listing

Fig. 1 scFv-Expression vector pLISC-SE containg the scFv-fragment.

Fig. 2 Dicistronic scFv-hinge-zipper expression vector pACKxFyJ.

Fig. 3 Functional ELISA;
The concentrations of the affinity purified proteins, measured by OD_{280} (vertical axis), refer to the molar number of binding sites per well (horizontal axis). The ELISA plates were coated with phosphocholine-BSA, and the purified phosphocholine-specific miniantibody-proteins were bound and detected by an anti-McPC603 antiserum.

(a) Comparison of various miniantibodies.
(b) Comparison of miniantibody scHLXc with ScFV and whole IgA.

Fig. 4 Functional Anti-lysozyme ELISA;
PC-affinity purified samples of coexpressed anti-PC-anti-lysozyme bispecific miniantibody. + and - on the horizontal axis means: plus inhibitor (+) and without inhibitor (-).

The attached sequence listing refers to sequence identity numbers (S.I.N.):

S.I.N. 1:	Whole nucleotid- and amino acid sequence of the pLISC-SE vector.
S.I.N. 2:	Gene cassette of intercalating GCN4-leucine zipper (nucleotid- and amino acid sequence).
S.I.N. 3:	Gene cassette encoding intercalating antiparallel helix-turn-helix (nucleotid- and amino acid sequence).
S.I.N. 4:	Gene cassette encoding intercalating jun-zipper and IgG3-hinge region.
S.I.N. 5:	Gene cassette encoding intercalating fos-zipper and IgG3-hinge region.
S.I.N. 6:	Gene cassette encoding intercalating jun-zipper and designed linker.
S.I.N. 7:	Gene cassette encoding intercalating fos-zipper and designed linker.

Example 1: Construction of vectors for secreted single-chain fragments, containing a restriction site for introducing genes for intercalating peptides.

Recombinant DNA-techniques were based on Sambrook et al. (1989, Molecular Cloning: A laboratory manual. Second edition. Cold Spring Harbor Laboratory, New York). Functional expression of the single-chain Fv fragments and the miniantibodies in E. coli JM83 was carried out with vectors similar to pASK-lisc (Skerra et al., 1991, Protein Eng. 4, 971). Site directed mutagenesis was directly performed in these vectors according to Kunkel et al. (1987, Meth. Enzymol. 154, 367-382) and Geisselsoder et al. (1987, Biotechniques 5, 786-791) using the helper phage M13K07 (Vieira & Messing, 1987, Meth. Enzymol. 153, 3-11). SDS-PAGE was carried out as described by Fling and Gregerson (1986, Anal. Biochem. 155, 83-88). Concentrations of affinity-purified proteins were measured by OD_{280} using calculated extinction coefficients (Gill & von Hippel, 1989, Anal. Biochem. 182, 319-326).A vector such as pASK40 (Skerra et al., 1991, Protein Eng. 4, 971) is used, which contains an origin of replication, a regulatable promotor, a bacterial signal sequence followed by a multiple cloning site, a transcription terminator and an origin for single stranded phages. The gene for the single-chain Fv fragment is designed as follows: The nucleotide sequence of a V_H domain is directly followed by a linker sequence encoding preferably about 15 residues, preferably of the sequence $(Gly_4Ser)_3$, followed directly by the sequence of the V_L domain. Alternatively, the sequence of the V_L domain may be directly followed by the sequence of the linker, followed by the sequence of the V_H domain.

If the antibody is of known sequence, the complete gene of the scFv fragment may be assembled from synthetic oligonucleotides. A detailed experimental procedure for such a gene synthesis of an antibody gene is e.g. given in Plückthun et al. (1987, Cold Spring Harbor Symp. Quant. Biol. 52, 105-112).

If the genes of the V_H and V_L domains are present in other vectors, the gene for the scFv fragment may be assembled from restriction fragments. For example, a restriction fragment encoding most of the V_H domain may be excised from another plasmid, and a fragment encoding most of the V_L domain may be excised from a plasmid. The remaining pieces of V_L and V_H and the linker for the scFv fragment can be provided by cassettes of synthetic oligonucleotides, which need to be ligated by standard methodology (Sambrook et al., 1989, literature cited). The mixture of fragments is ligated into the vector pASK40 or a similar plasmid containing a pair of suitable restriction sites.

If the genes of the antibody have not been cloned before, they may be directly obtained from the hybridoma cell producing the antibody by the polymerase chain reaction (PCR; PCR methodology is described in McPherson et al., 1991, PCR-A Practical Approach Oxford University Press, New York). Primers suitable for amplification of V_H and V_L domains have been given by Orlandi et al., 1989, Proc. Natl. Acad. Sci. USA 86, 3833-3837; Huse et al., 1989, Science 246, 1275-1281; Larrick et al., 1989, Bio-technology 7, 934-938. The methodology of obtaining mRNA from hybridoma is described in these references as well. The separate V_H and V_L genes may be cloned into separate vectors, and the scFv gene assembled according to the principles explained above.

If the ligated fragments do not result in a correct reading frame of the scFv fragment, a precise fusion with the signal sequence codons resident on the plasmid may be generated by site directed mutagenesis. The design of the oligonucleotides and the execution is possible for anyone skilled in the art.

The scFv expression plasmid so obtained contains the codons for a bacterial signal sequence, directly followed by the first variable domain (V_H or V_L), a linker and the second variable domain (V_L or V_H) under the control of a regulatable promotor.

At the 3' end of this genes, corresponding to the C-terminus of the scFv protein, a unique restriction site is introduced into the expression plasmid to allow insertion of cassettes coding for the intercalating peptides. The restriction site is introduced by site directed mutagenesis using the method of Kunkel (1987, Meth. Enzymol. 154, 367-382).

An example of the complete sequence of a suitable single-chain Fv expression plasmid pLISC-SE for receiving an intercalation peptide is shown in Fig. 1 and Sequence Identity No. (S.I.N.) 1 (see Squence Listing).

Example 2: Design and construction of a gene cassette encoding intercalating peptides of a leucine zipper.

The gene cassette, fitted with restriction sites to be compatible with the restriction site at the 3' end of the scFv fragment gene, must encode the sequence of a hinge (connection the scFv fragment to the intercalating peptide) and the intercalation peptide itself. The hinge region, may however also be obmitted.
As an example the sequence of the upper hinge region of mouse IgG3 (Dangl et al., 1988, EMBO J. 7, 1989-1994), followed by the sequence of the leucine zipper sequence of the yeast protein GCN4 (Oas et al., 1990, Biochemistry T29, 2891-2894), is back-translated into frequently used E. coli codons (S.I.N.: 2). Oligonucleotides are synthesized, and ligated into the vector pLISC-SE, previously digested with EcoRI and Hind III.

Example 3: Design and construction of a gene cassette encoding intercalating peptides of a four-helix bundle.

Analogous to Example 2, the sequence of the upper hinge region of mouse IgG3, followed by the sequence of the helix-turn-helix of a four helix bundle (Eisenberg et al., 1986, literature cited) is backtranslated into frequently used E. coli codons (S.I.N.: 3) . Oligonucleotides are synthesized, and ligated into the vector pLISC-SE, previously digested with Eco RI and Hind III.

Example 4: Design and construction two gene cassettes encoding intercalating peptides of a leucine zipper and their co-expression.

Analogous to Example 2, the sequence of the upper hinge region of mouse IgG3 followed by the sequence of the zipper sequence of the jun protein (O'Shea et al., 1992, literature cited), is backtranslated into frequently used E. coli codons (S.I.N.: 4). Oligonucleotides are synthesized, and ligated into the vector pLISC-SE, previously digested with EcoRI and Hind III.

In a parallel reaction, the sequence of the upper hinge region of mouse IgG3, followed by the sequence of the zipper sequence of the fos protein (O'Shea et al., 1992, Cell 68, 699-708), is backtranslated into frequently used E. coli codons (S.I.N.: 5). Oligonucleotides are synthesized, and ligated into the vector pLISC-SE, previously digested with Eco RI and Hind III.The two vectors thus each code for a different antibody scFv fragment, followed by a hinge peptide and a different leucine zipper peptide. To co-express the two scFv fragments, the whole scFv-hinge-zipper gene of the fos-containing product is excised from the vector as a Xba I-Hind III fragment and ligated into the vector, pLISC-SE-scFv-jun, containing already the scFv gene of the other antibody.
The newly obtained vector then expresses the $scFv_1$-$linker_1$-fos-zipper and the $scFv_2$-$linker_2$-jun-zipper from a single promoter as a dicistronic operon.

An improved sequence for the hinge region in the context of fos and jun zippers is given in S.I.N.: 6 and 7. This hinge is shorter and therefore not as susceptible to proteolysis. In cases, where the distance between the two binding sites is of less importance, such shortened hinges may be advantageous. In this case, the "tail" of the scFv fragment has been shortened and the EcoRI site, which receive the genes for the intercalating peptides has been moved four residues upstream.

Example 5: Purification of bivalent miniantibody from E. coli.

E. coli JM83, harboring a plasmid constructed as in examples II and III, are grown to an O.D. 550 of 0.5 and induced with IPTG at a final concentration of 1 mM. The cells are centrifuged, resuspended in BBS buffer (200 mM Na-borate, 160 mM NaCl, pH 8.0) and the suspension is passed through a French press. In these examples, a phosphorylcholine binding miniantibody is used. The miniantibody is purified by passage over a phosphorylcholine affinity chromatography as described (Chesebro and Metzger, 1972, Biochemistry 11, 766-771).

Example 6: Purification of a bispecific miniantibody from E. coli

E. coli JM83, harboring a plasmid constructed as in examples II and III and containing a dicistronic structural gene for two different scFv (Fig. 2), are grown to an O.D. 550 of 0.5 and induced with IPTG at a final concentration of 1mM. The cells are centrifuged, resuspended in BBS buffer (200 mM Na-borate, 160 mM NaCl, pH 8.0) and the suspension is passed through a French press.
In this example, a bispecific miniantibody is used containing both a specificity for phosphorylcholine as well as benzoylampicillin. The miniantibody is purified by passage over a phosphorylcholine affinity chromatography as described (Chesebro and Metzger, 1972, literature cited).

Example 7: Surface binding of bivalent miniantibodies

The ELISA-plates (Nunc, Macrosorp) were coated with 400 ~g/ml phosphocholine-BSA in PBS buffer (20 mM phosphate, pH 7.2, 115 mM NaCl). The hapten reagent was prepared from nitrophenyl phosphocholine (Sigma), which was reduced and diazotized essentially as described (Chesebro & Metzger, 1972, literature cited), and reacted by azo-coupling to BSA (Sigma) in borate-saline buffer (52.5 mM sodium borate, pH 9, 120 mM NaCl) at 4~C for 48 hours with subsequent dialysis against PBS. After blocking the non-coated plate surface with 5% skim milk (Nestle) in PBS buffer for at least 2 hours, the periplasmic extract or the purified protein was incubated in BBS buffer on the plate for 90 min at room temperature. After thorough washing (3 times), remaining functional antibody fragments were detected according standard procedures (Harlow & Lane, 1988, "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory, 555-592) with rabbit anti-McPC603 serum and anti-rabbit immunoglobulin linked to peroxidase (Sigma) according to Gallati (1979, Clin. Chem. Clin. Biochem. 17, 1-4).

An enormous gain in binding, and thus sensitivity, is observed for all miniantibody constructs, compared to the monomeric scFv fragment. This is consistent with the simultaneous binding of two or even more binding sites to the same surface. These avidity of the fusion protein scHLXc was comparable to the natural antibody McPC603, which could be detected with antigen-coated ELISA, while the monomeric scFv fragment could only be detected with hundred-fold higher concentrations (Fig. 3 a, b). All binding is nearly totally inhibitible with soluble hapten, except of the monomeric scFv fragment. The thermodynamic affinity of the natural antibody to soluble phosphocholine is about 1.6 \$ 10^5 M^{-1} and thus relatively weak (Metzger et al., 1971, Proceedings of the Ist Congress of Imunology. Academic Press, New York, pp. 253-267), and this is apparently not sufficient for a monomeric fragment-hapten complex to survive the repeated washing steps of a functional ELISA (Kemeny & Challacombe, 1988, "ELISA and other solid phase immunoassays", Wiley & Sons, New York).

Example 8: Surface binding of bifunctional miniantibodies

Coexpressed bifunctional miniantibodies recognizing phosphorylcholine with one arm and lysozyme with the other arm were purified by phosphocholine (PC) affinity chromatography and tested for lysozyme specificity. An ELISA-plate was coated with lysozyme, the ELISA was carried out as described in Example VII.Three different preparations show binding to the antigen-surface, which is completely inhibitible with soluble lysozyme (Fig. 4).

SEQUENCE LISTING:

SEQ ID NO: 1

```
ACCCGACACC ATCGAATGGC GCAAAACCTT TCGCGGTATG GCATGATAGC      50
GCCCGGAAGA GAGTCAATTC AGGGTGGTGA ATGTGAAACC AGTAACGTTA     100
TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG TTTCCCGCGT     150
GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAG     200
CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACTG     250
GCGGGCAAAC AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT     300
GCACGCGCCG TCGCAAATTG TCGCGGCGAT TAAATCTCGC GCCGATCAAC     350
TGGGTGCCAG CTGTGTGGTG TCGATGGTAG AACGAAGCGG CGTCGAAGCC     400
TGTAAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA GTGGGCTGAT     450
CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT     500
GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC     550
AACAGTATTA TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA     600
TCTGGTCGCA TTGGGTCACC AGCAAATCGC GCTGTTAGCG GGCCCATTAA     650
GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG GCTGGCATAA ATATCTCACT     700
CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT GGAGTGCCAT     750
GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA     800
CZGCGATGCT GGTTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC     850
ATTACCGAGT CCGGGCTGCG CGTTGGTGCG GATGTCTCGG TAGTGGGATA     900
CGCAGATACC GAAGACAGCT CATGTTATAT CCCGCCGTTA ACCACCATCA     950
AACAGGATTT TCGCCTGCTG GGGCAAACCA GCGTGGACCG CTTGCTGCAA    1000
CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC CCGTCTCACT    1050
GGTGAAAAGA AAAACCACCC TGGCGCCCAA TACGCAAACC GCCTCTCCCC    1100
GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG    1150
GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CTCACTCATT    1200
AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCGTAT GTTGTGTGGA    1250
ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG ACCATGATTA    1300
CGAATTTCTA GATAACGAGG GCAAAAA---ATG AAA AAG ACA GCT ATC    1345
                                Met Lys Lys Thr Ala Ile
                                1               5

GCG ATT GCA GTG GCA CTG GCT GGT TTC GCT ACC GTA GCG CAG    1387
Ala Ile Ala Val Ala Leu Ala Gly Phe Ala Thr Val Ala Gln
            10              15                  20

GCC GAA GTT AAA CTG GTA GAG TCT GGT GGT GGT CTG GTA CAG    1429
Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln
                25                  30

CCG GGT GGA TCC CTG CGT CTG TCT TGC GCT ACC TCA GGT TTC    1471
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe
35                  40                  45

ACC TTC TCT GAC TTC TAC ATG GAG TGG GTA CGT CAG CCC CCG    1513
Thr Phe Ser Asp Phe Tyr Met Glu Trp Val Arg Gln Pro Pro
    50                  55                  60

GGT AAA CGT CTC GAG TGG ATC GCA GCT AGC CGT AAC AAA GGT    1555
Gly Lys Arg Leu Glu Trp Ile Ala Ala Ser Arg Asn Lys Gly
    65                  70                  75

AAC AAG TAT ACC ACC GAA TAC AGC GCT TCT GTT AAA GGT CGT    1597
Asn Lys Tyr Thr Thr Glu Tyr Ser Ala Ser Val Lys Gly Arg
            80                  85                  90
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TTC ATC GTT TCT CGT GAC ACT AGT CAA TCG ATC CTG TAC CTG      1639
Phe Ile Val Ser Arg Asp Thr Ser Gln Ser Ile Leu Tyr Leu
                95                  100
CAG ATG AAT GCA TTG CGT GCT GAA GAC ACC GCT ATC TAC TAC      1681
Gln Met Asn Ala Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr
105                 110                 115
TGC GCG CGT AAC TAC TAT GGC AGC ACT TGG TAC TTC GAC GTT      1723
Cys Ala Arg Asn Tyr Tyr Gly Ser Thr Trp Tyr Phe Asp Val
    120                 125                 130
TGG GGT GCA GGT ACC ACC GTT ACC GTT TCT TCT GGT GGT GGT      1765
Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
        135                 140                 145
GGT TCT GGT GGT GGT GGT TCT GGT GGT GGT GGT TCT GAT ATC      1807
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
            150                 155                 160
GTT ATG ACC CAG TCT CCG AGC TCT CTG TCT GTA TCT GCA GGT      1849
Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly
                165                 170
GAA CGT GTT ACC ATG TCT TGC AAA TCT TCT CAG TCT CTG CTG      1891
Glu Arg Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
175                 180                 185
AAC TCT GGT AAC CAG AAA AAC TTC CTG GCG TGG TAT CAG CAA      1933
Asn Ser Gly Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln
    190                 195                 200
AAG CCT GGC CAA CCG CCG AAA CTG CTG ATC TAC GGT GCG TCG      1975
Lsy Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser
        205                 210                 215
ACC CGT GAA TCT GGT GTT CCG GAC CGT TTT ACC GGT AGC GGT      2017
Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
            220                 225                 230
AGC GGT ACC GAC TTC ACT CTG ACC ATC TCT TCT GTA CAG GCT      2059
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala
                235                 240
GAA GAT CTG GCT GTT TAC TAC TGT CAA AAC GAC CAC TCT TAC      2101
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp His Ser Tyr
245                 250                 255
CCG CTG ACC TTT GGC GCC GGC ACC AAA CTG GAA CTG AAG CGC      2143
Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
    260                 265                 270
GCT AAC GGT GAA TTC-TGATAAGCTTGA CCTGTGAAGT GAAAAATGGC       2190
Ala Asn Gly Glu Phe *
        275
GCACATTGTG CGACATTTTT TTTGTCTGCC GTTTACCGCT ACTGCGTCAC       2240
GGATCCCCAC GCGCCCTGTA GCGGCGCATT AAGCGCGGCG GGTGTGGTGG       2290
TTACGCGCAG CGTGACCGCT ACACTTGCCA GCGCCCTAGC GCCCGCTCCT       2340
TTCGCTTTCT TCCCTTCCTT TCTCGCCACG TTCGCCGGCT TTCCCCGTCA       2390
AGCTCTAAAT CGGGGCATCC CTTTAGGGTT CCGATTTAGT GCTTTACGGC       2440
ACCTCGACCC CAAAAAACTT GATTAGGGTG ATGGTTCACG TAGTGGGCCA       2490
TCGCCCTGAT AGACGGTTTT TCGCCCTTTG ACGTTGGAGT CCACGTTCTT       2540
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TCTATTCTTT TGATTTATAA GGGATTTTGC CGATTTCGGC CTATTGGTTA       2640
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CTTTTGCTGG CCTTTTGCTC ACATG 4515

SEQ ID NO: 2

```
GGT GAA TTC CCC AAA CCT AGT ACT CCC CCT GGC AGC AGC CGC ATG 45
Gly Glu Phe Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met
1    ↑          5                   10                  15
            └--------- IgG3-hinge ------------------┘ └------
AAA CAG CTG GAA GAT AAA GTT GAA GAG CTT CTT TCG AAA AAC TAC 90
Lys Gln Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr
                20                  25                  30
------------------------ GCN4-zipper ----------------------
CAC CTC GAA AAT GAA GTT GCG CGC CTC AAA AAA CTT GTT GGT GAA 135
His Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu
                35                  40                  45
-----------------------------------------------------------
CGC TGATAAGCTT GAC                                          151
Arg      ↑
---┘stop
```

SEQ ID NO: 3

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GGT GAA TTC CCC AAA CCT AGC ACC CCC CCT GGC AGC AGT GGT GAA 45
Gly Glu Phe Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Gly Glu
1    ↑          5                   10                  15
            └-------- IgG3-hinge --------------------┘ └------
CTG GAA GAG CTG CTT AAG CAT CTT AAA GAA CTT CTG AAG GGC CCC 90
Leu Glu Glu Leu Leu Lys His Leu Lys Glu Leu Leu Lys Gly Pro
                20                  25                  30
------------- bundle-helix A ----------------------------┘ └--
CGC AAA GGC GAA CTC GAG GAA CTG CTG AAA CAT CTG AAG GAG CTG 135
Arg Lys Gly Glu Leu Glu Glu Leu Leu Lys His Leu Lys Glu Leu
                35                  40                  45
-turn--┘ └--------------- bundle-helix B -------------------
CTT AAA GGT GAA TTC TGATAAGCTT GACCTGTGAA GTGAAAAAAT G      191
Leu Lys Gly Glu Phe
            ↑   50     ↑
-------------------┘stop
```

SEQ ID NO: 4

```
GGT GAA TTC CCC AAA CCT AGT ACT CCC CCT GGC AGC AGC CGT ATC  45
Gly Glu Phe Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Ile
 1   ↑           5                   10                  15
            L------IgG3-hinge-----------------------J L------
GCT CGT CTC GAG GAA AAA GTT AAA ACC CTG AAA GCT CAG AAC TCC  90
Ala Arg Leu Glu Glu Lys Val Lys Thr Leu Lys Ala Gln Asn Ser
                 20                  25                  30
--------------------- jun-zipper -------------------------
GAA CTG GCT TCC ACC GCT AAC ATG CTG CGT GAA CAG GTT GCT CAG 135
Glu Leu Ala Ser Thr Ala Asn Met Leu Arg Glu Gln Val Ala Gln
                 35                  40                  45
--------------------- jun-zipper -------------------------
CTG AAA CAG AAA GTT ATG AAC TAC TGATAAGCTT GACCTGTGAA G     180
Leu Lys Gln Lys Val Met Asn Tyr       ↑
                 50
-----------------------------J stop
```

SEQ ID NO: 5

```
GGT GAA TTC CCC AAA CCT AGT ACT CCC CCT GGC AGC AGC CTG ACC  45
Gly Glu Phe Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Leu Thr
 1   ↑           5                   10                  15
            L------------- IgG3-hinge ---------------J L-----
GAC ACC CTG CAG GCT GAA ACC GAC CAG CTG GAA GAC AAA AAA TCC  90
Asp Thr Leu Gln Ala Glu Thr Asp Gln Leu Glu Asp Lys Lys Ser
                 20                  25                  30
----------------------- fos-zipper -----------------------
GCT CTG CAG ACC GAA ATC GCT AAC CTG CTG AAA GAA AAA GAA AAA 135
Ala Leu Gln Thr Glu Ile Ala Asn Leu Leu Lys Glu Lys Glu Lys
                 35                  40                  45
----------------------- fos-zipper -----------------------
CTG GAA TTT ATC CTG GCT GCT TAC TGATAAGCTT GACCTGTGAA G     180
Leu Glu Phe Ile Leu Ala Ala Tyr       ↑
                 50
-----------------------------J stop
```

SEQ ID NO: 6

```
GGT GAA TTC CCG TCT GGT AAC GAA GCT CGT ATC GCT CGT CTC GAG  45
Gly Glu Phe Pro Ser Gly Asn Glu Ala Arg Ile Ala Arg Leu Glu
 1   ↑           5                  10                  15
            L---- linker ----------┘ L----------------------
GAA AAA GTT AAA ACC CTG AAA GCT CAG AAC TCC GAA CTG GCT TCC  90
Glu Lys Val Lys Thr Leu Lys Ala Gln Asn Ser Glu Leu Ala Ser
                 20                  25                  30
------------------------ jun-zipper -----------------------
ACC GCT AAC ATG CTG CGT GAA CAG GTT GCT CAG CTG AAA CAG AAA  135
Thr Ala Asn Met Leu Arg Glu Gln Val Ala Gln Leu Lys Gln Lys
                 35                  40                  45
------------------------ jun-zipper -----------------------
GTT ATG AAC TAC TGATAAGCTT GACCTGTGAA GTGAAAAATG GCG         180
Val Met Asn Tyr        ↑
---------------┘ stop
```

SEQ ID NO: 7

```
GGT GAA TTC GGT CCG TCT GGT AAC GAA CTG ACC GAC ACC CTG CAG  45
Gly Glu Phe Gly Pro Ser Gly Asn Glu Leu Thr Asp Thr Leu Gln
 1   ↑           5                  10                  15
            L--- linker -----------┘ L----- fos-zipper -------
GCT GAA ACC GAC CAG CTG GAA GAC AAA AAA TCC GCT CTG CAG ACC  90
Ala Glu Thr Asp Gln Leu Glu Asp Lys Lys Ser Ala Leu Gln Thr
                 20                  25                  30
------------------- fos-zipper ----------------------------
GAA ATC GCT AAC CTG CTG AAA GAA AAA GAA AAA CTG GAA TTT ATC  135
Glu Ile Ala Asn Leu Leu Lys Glu Lys Glu Lys Leu Glu Phe Ile
                 35                  40                  45
------------------- fos-zipper ----------------------------
CTG GCT GCT TAC TGATAAGCTT GACCTGTGAA GTGAAAAATGGCG          180
Leu Ala Ala Tyr        ↑
---------------┘ stop
```

Claims

1. Monomeric antibody-fragment fusion protein consisting of a Fv-fragment of an antibody and a peptide which is capable to dimerize with another peptide by noncovalent interaction.

2. Monomer according to claim 1 characterized in that the Fv-fragment is a single chain fragment.

3. Monomer according to claim 1 or 2 characterized in that the interactive peptide consists of 10 to 50, preferrably 10 to 30 amino acids.

4. Monomer according to one of the claims 1 or 3 characterized in that the peptide consists of at least one helix.

5. Monomer according to claim 4 characterized in that the helix peptide consists of a helix, a turn and another helix.

6. Monomer according to claims 4 characterized in that the peptide contains a leucine zipper molecule, having several repeating amino acids, in which every seventh amino acid is a leucine.

7. Monomer according to claims 4 characterized in that the peptide bears charged residues.

8. Monomer according one of the claims 1 to 7 characterized in that a linking peptide is between the Fv-fragment and the peptide.

9. Monomer according to claim 8 characterized in that the linking peptide is a hinge region sequence of an antibody or a fragment thereof.

10. Process for preparation of a monomeric antibody fusion protein as defined in claims 1 to 9, characterized in that the genes coding for the Fv-fragment, the interactive peptide and, if desired, the linking peptide are cloned into one expression plasmid, a host cell is transformed with said expression plasmid and cultivated in a nutrient solution, and the monomeric fusion protein is expressed in the cell or secreted into the medium.

11. Process according to claim 10 characterized in that the host cell is E. coli.

12. Dimeric fusion protein consisting of two monomeric fusion proteins, wherein the linkage of the monomeric units bases on noncovalent interaction of identical or different peptides, characterized in that at least one monomeric unit is an antibody-fragment fusion protein as defined in claims 1 to 9.

13. Dimeric fusion protein according to claim 12 wherein the interactive peptides are the same.

14. Dimer according to claim 12 or 13 characterized in that the second monomeric unit is an antibody-fragment fusion protein as defined in claims 1 to 9 having different specificity.

15. Dimer according to claim 12 or 13 characterized in that the second monomeric unit is a fusion protein as defined in claims 1 to 9, wherein the antibody-fragment (Fv) is replaced by a non-antibody protein or peptide.

16. Dimer according to claim 15 characterized in that the protein or peptide is a toxin, a chelator peptide, a metal binding protein or an enzyme, or has the corresponding specific binding site.

17. Dimer according to claim 15 characterized in that the protein or peptide has a T-cell-, or a T-cell fragment specific binding site.

18. Dimer according to one of the claims 12 to 17, wherein another protein is fused at the C-terminus of one or both of the intercalating peptides.

19. Dimer according to claim 18, wherein the fused protein is a toxin, a chelator peptide, a metal binding protein or an enzyme, or has the corresponding specific binding site, or has a T-cell (fragment) specific binding site.

20. Process for preparation of a dimeric fusion proteinas defined in claims 12 to 19 characterized in that the genes coding for the complete monomeric fusion proteins or parts of it are cloned at least into one expression plasmid, a host cell is transformed with said expression plasmid(s) and cultivated in a nutrient solution, and either the complete dimeric fusion protein is expressed in the cell or into the medium, or the monomeric fusion proteins are separately expressed and the noncovalent linkage between the two monomeric units is performed in the medium or in vitro, and in the case that only parts of the fusion proteins were cloned protein engineering steps are additionally performed.

21. Process according to claim 20 characterized in that the gene coding for the first monomeric fusion protein is cloned into a first expression plasmid, and the gene coding for the second monomeric fusion protein is cloned into a second expression plasmid.

22. Process according to claim 20 characterized in that the noncovalent linkage between the monomeric units forming the dimeric fusion protein is performed in vitro.

23. Process according to one of the claims 20 to 22 characterized in that the host cell is E. coli.

24. Construction kit for preparation of seletive dimers of antibody-fragment fusion proteins as defined in claims 12 to 19 containing(a) a monomeric antibody-fragment fusion protein as defined in claims 1 to 8, and (b) a second

monomeric fusion protein as defined in (a), wherein the antibody fragment has the same or another antigen specificity, or wherein the antibody fragment unit is replaced by a non-antibody protein/peptide.

Patentansprüche

1. Monomeres Antikörperfragment-Fusionsprotein bestehend aus einem Fv-Fragment eines Antikörpers und eines Peptids, das zur Dimerisierung mit einem anderen Peptid durch nichtkovalente Wechselwirkung fähig ist.

2. Monomer gemäß Anspruch 1, dadurch gekennzeichnet, daß es sich bei dem Fv-Fragment um ein Einzelkettenfragment handelt.

3. Monomer gemäß Anspruch 1 oder 2, dadurch gekennzeichnet, daß das die Wechselwirkung eingehende Peptid aus 10 bis 50, vorzugsweise 10 bis 30, Aminosäuren besteht.

4. Monomer gemäß einem der Ansprüche 1 oder 3, dadurch gekennzeichnet, daß das Peptid aus zumindest einer Helix besteht.

5. Monomer gemäß Anspruch 4, dadurch gekennzeichnet, daß das Helixpeptid aus einer Helix, einer Wendung und einer weiteren Helix besteht.

6. Monomer gemäß Anspruch 4, dadurch gekennzeichnet, daß das Peptid ein Leucin-Zippermolekül mit mehreren repetetiven Aminosäuren enthält, wobei es sich bei jeder siebten Aminosäure um ein Leucin handelt.

7. Monomer gemäß Anspruch 4, dadurch gekennzeichnet, daß das Peptid geladene Reste enthält.

8. Monomer gemäß einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, daß sich zwischen dem Fv-Fragment und dem Peptid ein Linkerpeptid befindet.

9. Monomer gemäß Anspruch 8, dadurch gekennzeichnet, daß es sich bei dem Linkerpeptid um eine Sequenz der Hinge-Region eines Antikörpers oder Antikörperfragments handelt.

10. Verfahren zur Herstellung eines monomeren Antikörperfusionsproteins nach Ansprüchen 1 bis 9, dadurch gekennzeichnet, daß die für das Fv-Fragment, das die Wechselwirkung eingehende Peptid und gewünschtenfalls das Linkerpeptid kodierenden Gene in ein Expressionsplasmid kloniert werden, eine Wirtszelle mit diesem Expressionsplasmid transformiert und in einer Nährlösung kultiviert wird und das monomere Fusionsprotein in die Zelle exprimiert oder in das Medium sezerniert wird.

11. Verfahren gemäß Anspruch 10, dadurch gekennzeichnet, daß es sich bei der Wirtszelle um E. coli handelt.

12. Dimeres Fusionsprotein bestehend aus zwei monomeren Fusionsproteinen, wobei die Verbindung der monomeren Einheiten auf nichtkovalenter Wechselwirkung identischer oder verschiedener Peptide basiert, dadurch gekennzeichnet, daß es sich bei zumindest einer monomeren Einheit um ein Antikörperfragment-Fusionsprotein nach Ansprüchen 1 bis 9 handelt.

13. Dimeres Fusionsprotein gemäß Anspruch 12, wobei die eine Wechselwirkung eingehenden Peptide identisch sind.

14. Dimer gemäß Anspruch 12 oder 13, dadurch gekennzeichnet, daß es sich bei der zweiten monomeren Einheit um ein Antikörperfragment-Fusionsprotein nach den Ansprüchen 1 bis 9 mit unterschiedlicher Spezifität handelt.

15. Dimer gemäß Anspruch 12 oder 13, dadurch gekennzeichnet, daß es sich bei der zweiten monomeren Einheit um ein Fusionsprotein nach Ansprüchen 1 bis 9 handelt, wobei das Antikörperfragment (Fv) durch ein Nichtantikörperprotein oder -peptid ersetzt ist.

16. Dimer gemäß Anspruch 15, dadurch gekennzeichnet, daß es sich bei dem Protein oder Peptid um ein Toxin, ein Komplexbildnerpeptid, ein metallbindendes Protein oder ein Enzym handelt, oder daß es über die entsprechende spezifische Bindungsstelle verfügt.

17. Dimer gemäß Anspruch 15, dadurch gekennzeichnet, daß das Protein oder Peptid über eine spezifische Bindungsstelle für T-Zellen oder T-Zellfragment verfügt.

18. Dimer gemäß einem der Ansprüche 12 bis 17, wobei ein weiteres Protein mit dem C-Terminus von einem oder beiden interkalierenden Peptiden fusioniert ist.

19. Dimer gemäß Anspruch 18, wobei es sich bei dem fusionierten Protein um ein Toxin, ein Komplexbildnerpeptid, ein metallbindendes Protein oder ein Enzym handelt oder wobei dieses über die entsprechende spezifische Bindungsstelle oder über eine T-Zellen- oder T-Zellfragment-spezifische Bindungsstelle verfügt.

20. Verfahren zur Herstellung eines dimeren Fusionsproteins nach Ansprüchen 12 bis 19, dadurch gekennzeichnet, daß die für das vollständige monomere Fusionsprotein bzw. für dessen Teile codierenden Gene in mindestens ein Expressionsplasmid kloniert werden, eine Wirtszelle mit diesem Expressionsplasmid bzw. diesen Expressionsplasmiden transformiert und in einer Nährlösung kultiviert wird, und entweder das vollständige dimere Fusionsprotein in die Zelle oder in das Medium exprimiert wird, oder die monomeren Fusionsproteine getrennt exprimiert werden und die nichtkovalente Bindung zwischen den beiden monomeren Einheiten in dem Medium oder in vitro vorgenommen wird, und daß, falls nur Teile der Fusionsproteine kloniert wurden, zusätzlich protein-technologische Schritte vorgenommen werden.

21. Verfahren gemäß Anspruch 20, dadurch gekennzeichnet, daß das für das erste monomere Fusionsprotein codierende Gen in ein erstes Expressionsplasmid kloniert wird und das für das zweite monomere Fusionsprotein codierende Gen in ein zweites Expressionsplasmid kloniert wird.

22. Verfahren gemäß Anspruch 20, dadurch gekennzeichnet, daß die nichtkovalente Bindung zwischen den das dimere Fusionsprotein bildenden monomeren Einheiten in vitro durchgeführt wird.

23. Verfahren gemäß einem der Ansprüche 20 bis 22, dadurch gekennzeichnet, daß es sich bei der Wirtszelle um E. coli handelt.

24. Konstruktionsbesteck zur Herstellung von selektiven Dimeren von Antikörperfragment-Fusionsproteinen nach Ansprüchen 12 bis 19 mit (a) einem monomeren Antikörperfragment-Fusionsprotein nach Ansprüchen 1 bis 8 und (b) einem zweiten monomeren Fusionsprotein nach (a), wobei das Antikörperfragment die gleiche oder eine andere Antigen-Spezifität aufweist, oder wobei die Antikörperfragment-Einheit durch ein Nichtantikörper-Protein/Peptid ersetzt ist.

Revendications

1. Protéine de fusion à fragment d'anticorps monomérique consistant en un fragment Fv d'un anticorps et en un peptide qui est capable de se dimériser avec un autre peptide par interaction non covalente.

2. Monomère selon la revendication 1, caractérisé en ce que le fragment Fv est un fragment monocaténaire.

3. Monomère selon la revendication 1 ou 2, caractérisé en ce que le peptide interactif consiste en 10 à 50, de préférence 10 à 30 acides aminés.

4. Monomère selon l'une des revendications 1 ou 3, caractérisé en ce que le peptide se compose d'au moins une hélice.

5. Monomère selon la revendication 4, caractérisé en ce que le peptide en hélice se compose d'une hélice, d'un coude et d'une autre hélice.

6. Monomère selon la revendication 4, caractérisé en ce que le peptide contient une molécule de fermeture ("zipper") à leucine, ayant plusieurs acides aminés répétés, dans laquelle un acide aminé sur sept est une leucine.

7. Monomère selon la revendication 4, caractérisé en ce que le peptide porte des résidus chargés.

8. Monomère selon l'une des revendications 1 à 7, caractérisé en ce qu'un peptide de liaison se trouve entre le

fragment Fv et le peptide.

9. Monomère selon la revendication 8, caractérisé en ce que le peptide de liaison est une séquence de région charnière d'un anticorps ou d'un de ses fragments.

10. Procédé de préparation d'une protéine de fusion à anticorps monomérique telle que définie dans les revendications 1 à 9, caractérisé en ce qu'on clone les gènes codant pour le fragment Fv, le peptide interactif et, si on le désire, le peptide de liaison, dans un plasmide d'expression, en ce qu'on transforme une cellule hôte avec ledit plasmide d'expression et en ce qu'on la cultive dans une solution nutritive, et en ce qu'on exprime la protéine de fusion monomérique dans la cellule ou en ce qu'on la sécrète dans le milieu.

11. Procédé selon la revendication 10, caractérisé en ce que la cellule hôte est E. coli.

12. Protéine de fusion dimérique consistant en deux protéines de fusion monomériques, dans laquelle la liaison des unités monomériques se fonde sur l'interaction non covalente de peptides identiques ou différents, caractérisée en ce qu'au moins une unité monomérique est une protéine de fusion à fragment d'anticorps telle que définie dans les revendications 1 à 9.

13. Protéine de fusion dimérique selon la revendication 12 dans laquelle les peptides interactifs sont les mêmes.

14. Dimère selon la revendication 12 ou 13, caractérisé en ce que la seconde unité monomérique est une protéine de fusion à fragment d'anticorps telle que définie dans les revendications 1 à 9 ayant une spécificité différente.

15. Dimère selon la revendication 12 ou 13, caractérisé en ce que la seconde unité monomérique est une protéine de fusion telle que définie dans les revendication 1 à 9, dans laquelle le fragment d'anticorps (Fv) est remplacé par une protéine ou un peptide non-anticorps.

16. Dimère selon la revendication 15, caractérisé en ce que la protéine ou le peptide est une toxine, un peptide chélateur, une protéine de liaison à un métal ou une enzyme, ou a le site de liaison spécifique correspondant.

17. Dimère selon la revendication 15, caractérisé en ce que la protéine ou le peptide a un site de liaison spécifique à une cellule T, ou à un fragment de cellule T.

18. Dimère selon l'une des revendications 12 à 17, dans lequel une autre protéine est fusionnée à l'extrémité C-terminale d'un ou des deux peptides intercalants.

19. Dimère selon la revendication 18, dans lequel la protéine fusionnée est une toxine, un peptide chélateur, une protéine de liaison à un métal ou une enzyme, ou a le site de liaison spécifique corresponddant, ou a un site de liaison spécifique à une cellule T (fragment).

20. Procédé de préparation d'une protéine de fusion dimérique définie dans les revendications 12 à 19, caractérisé en ce qu'on clone les gènes codant pour les protéines de fusion monomériques complètes ou des parties de ces protéines au moins dans un plasmide d'expression, en ce qu'on transforme une cellule hôte avec le(s)dit(s) plasmide(s) d'expression et qu'on la cultive dans une solution nutritive, et soit qu'on exprime la protéine de fusion dimérique complète dans la cellule ou dans le milieu, soit qu'on exprime séparément les protéines de fusion monomériques et qu'on effectue la liaison non covalente entre les deux unités monomériques dans le milieu ou in vitro, et dans le cas où l'on ne clone que des parties des protéines de fusion, on effectue en outre des étapes de génie protéique.

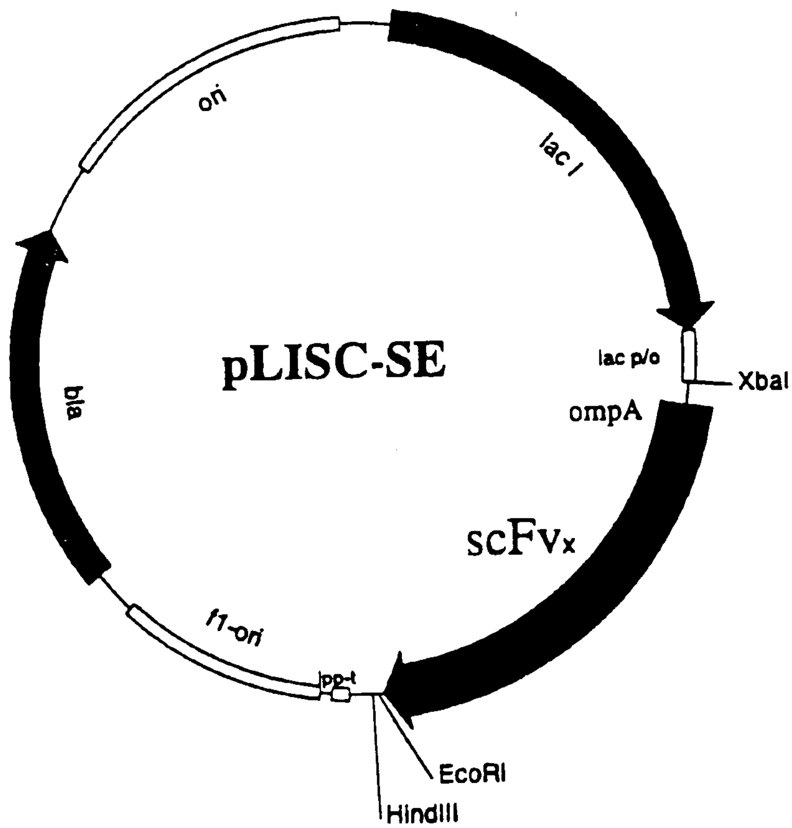
21. Procédé selon la revendication 20, caractérisé en ce qu'on clone le gène codant pour la première protéine de fusion monomérique dans un premier plasmide d'expression, et en ce qu'on clone le gène codant pour la seconde protéine de fusion monomérique dans un second plasmide d'expression.

22. Procédé selon la revendication 20, caractérisé en ce qu'on effectue in vitro la liaison non covalente entre les unités monomériques formant la protéine de fusion dimérique.

23. Procédé selon l'une des revendications 20 à 22, caractérisé en ce que la cellule hôte est E. coli.

24. Trousse de construction pour la préparation de dimères sélectifs de protéines de fusion à fragments d'anticorps telles que définies dans les revendications 12 à 19, contenant (a) une protéine de fusion à fragment d'anticorps monomérique telle que définie dans les revendications 1 à 8, et (b) une seconde protéine de fusion monomérique telle définie en (a), dans laquelle le fragment d'anticorps a une spécificité antigénique identique ou différente, ou bien dans laquelle l'unité fragment d'anticorps est remplacé par une protéine/un peptide non-anticorps.

FIG. 1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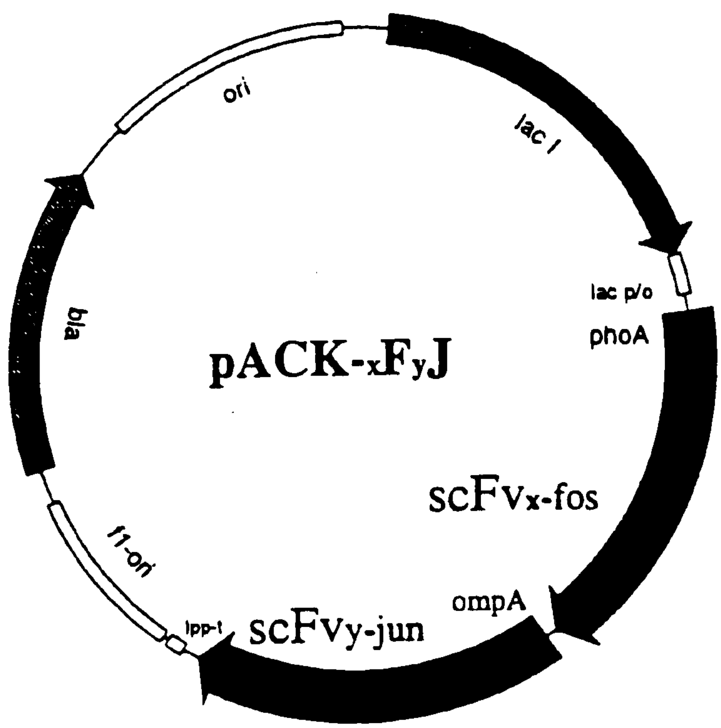
ompA
scFvx
bla
f1-ori
lpp-t
EcoRI
HindIII

FIG. 2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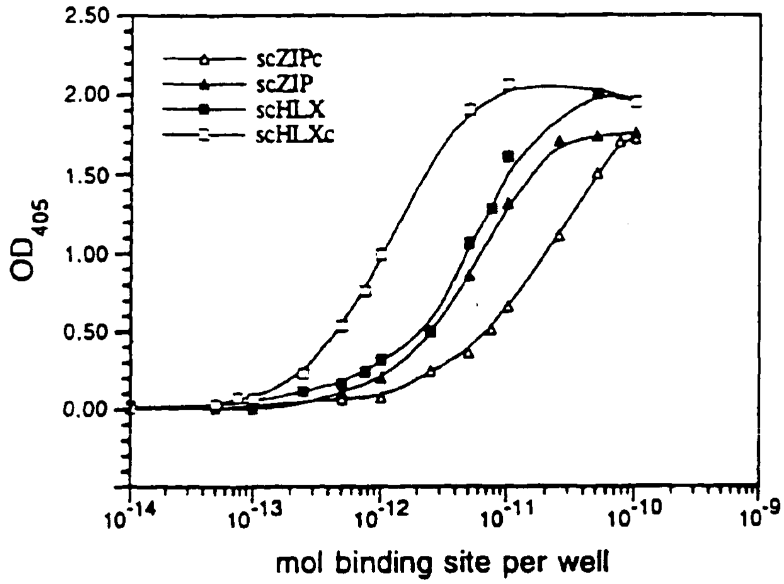
lac I
lac p/o
phoA
bla
pACK-xFyJ
scFvx-fos
f1-ori
ompA
lpp-t
scFvy-jun

Fig. 3 a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scZIP
scHLX
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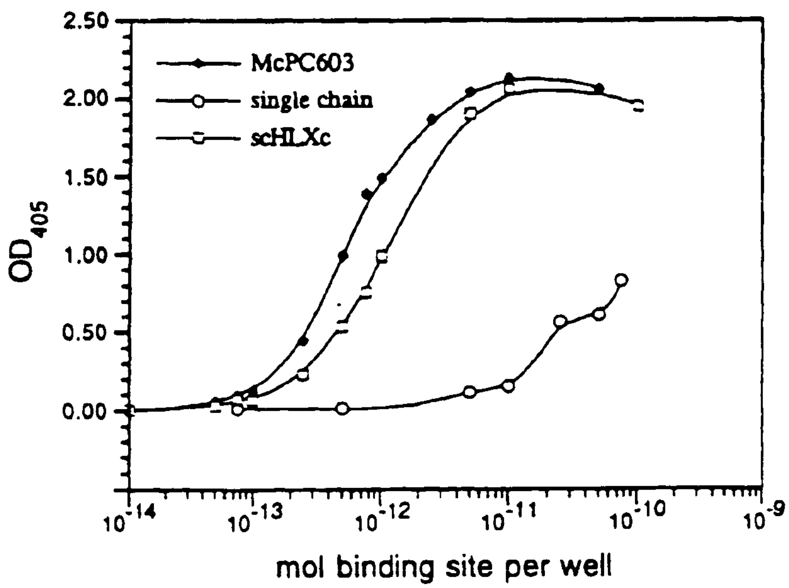
2.00
1.50
1.00
0.50
0.00
OD405
10-14
10-13
10-12
10-11
10-10
10-9
mol binding site per well

Fig. 3 b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scHLXc
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2.00
1.50
1.00
0.50
0.00
OD405
10-14
10-13
10-12
10-11
10-10
10-9
mol binding site per well

Fig. 4